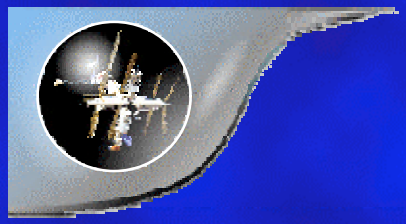
STATE SCIENTIFIC CENTER RF -
INSTITUTE FOR BIOMEDICAL PROBLEMS,
MOSCOW, RUSSIA

REVIEW OF THE KNOWLEDGE OF MICROBIAL CONTAMINATION OF THE RUSSIAN MANNED SPACECRAFT

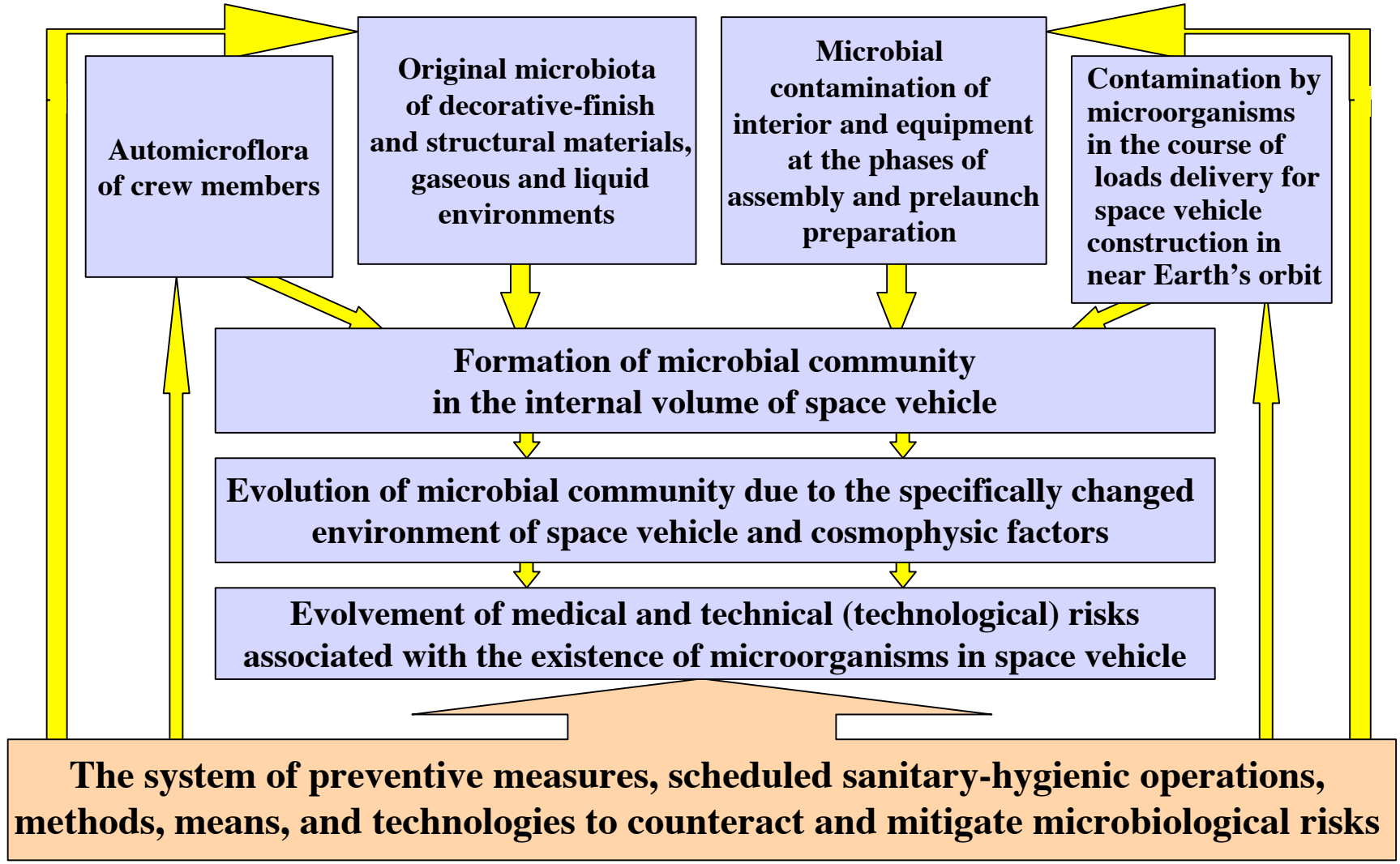
Natalja Novikova



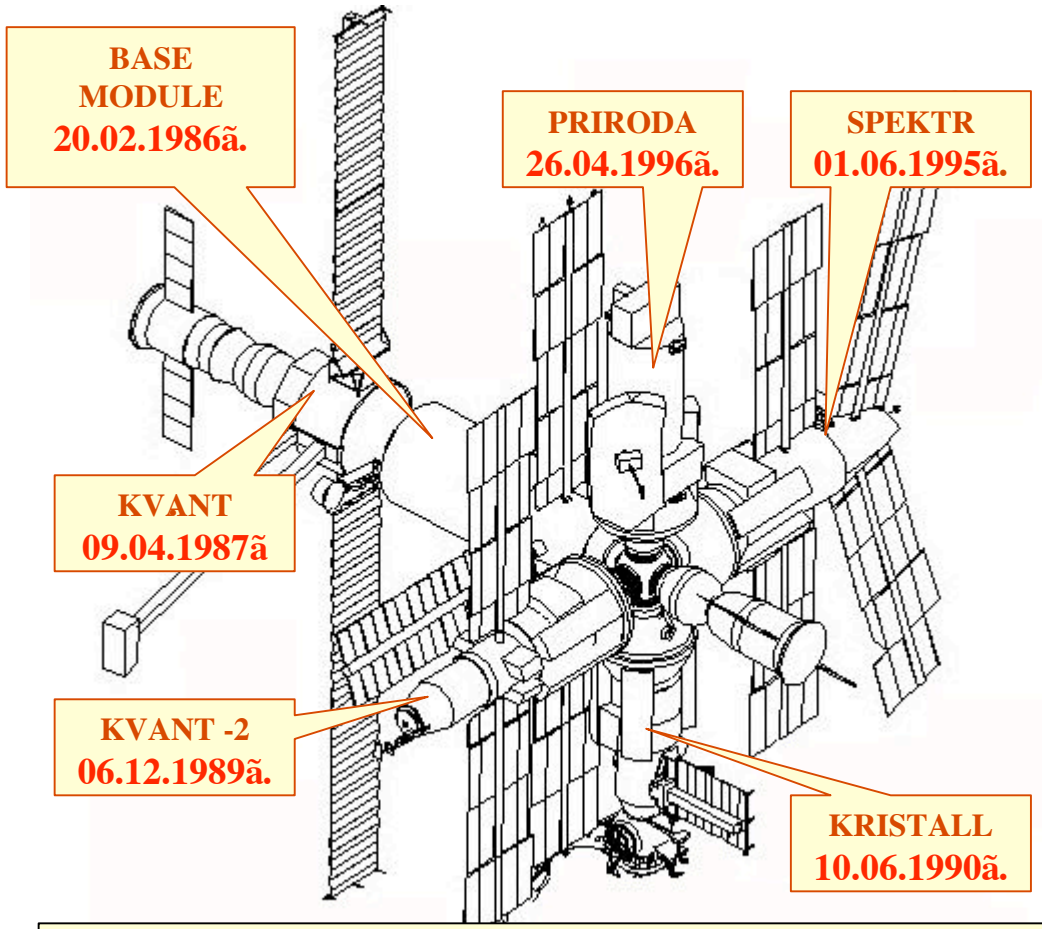
THEMES OF THE REPORT

- GENERAL CHARACTERISTIC OF THE MICROBIAL COMMUNITY IN THE HABITABLE COMPARTMENTS OF PILOTED SPACE VEHICLES.
- MICROFLORA OF INDIVIDUAL COMPONENTS OF SPACE VEHICLE ENVIRONMENT.
- MICROBIOLOGICAL RISKS IN EXTENDED SPACE MISSION.
- PECULIARITIES OF EVOLUTION OF MICROFLORA UNDER THE SPACE CONDITIONS.
- BASIC PRINCIPLES OF MICROBIAL MONITORING OF THE ORBITAL STATION ENVIRONMENT IN VIEW OF MANY YEARS OF OPERATION.
- THE PRIORITIZED DIRECTIONS OF PERFECTION OF METHODS AND MEANS OF MICROBIOLOGICAL SAFETY IN LONG-OPERATING SPACE VEHICLES.

THE MICROBIOLOGICAL FACTOR OF SPACE FLIGHT



OPERATION OF ORBITAL COMPLEX "MIR"
1986 - 2000



Russian experiments

- Microbiological monitoring
- Ecosphere
- Bioresistance

Russian/US experiments

- Air quality
- Microflora of surfaces
- Mir potable water

Multiyear dynamics was investigated in:

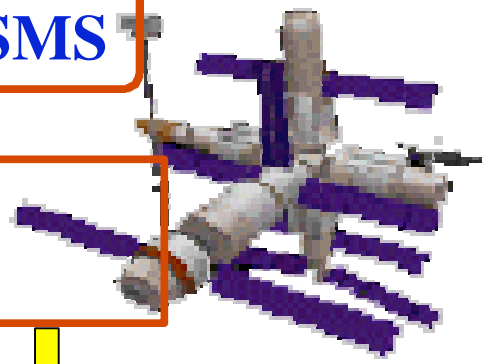
- air samples taken in 12 Mir locations
- samples of stock and regenerated water, and air condensate
- smears of interior and equipment make in 85 locations
- smears from specified decorative-finish and structural materials
- components of systems, and units.

Identification of "space" strains of microorganismsè was performed using microbial analyzer VITEK-60 (France)

Results of the investigations laid the ground for automated data base (5 810 filings, 662 726 Kb)

STRUCTURE OF THE "MIR" MICROECOSPHERE

234 SPECIA OF MICROORGANISMS



BACTERIA
40 GENES
108 SPECIA

FUNGI
25 GENES
126 SPECIA

Opportunistic pathogenic bacteria (IV groups of pathogenicity)

- Staphylococcus sp. (S.aureus)
- Streptococcus sp.
- Escherichia coli
- Proteus sp.
- Serratia marcescens
- Hafnia alvei
- Flavobacterium meningosepticum
- Klebsiella pneumoniae
- Bacillus cereus

Fungi "pathogenic saprophytes"

- Aspergillus flavus
- Aspergillus fumigatus
- Aspergillus niger
- Penicillium crustosum
- Geotrichum candidum
- Candida parapsilosis
- Candida sp.

Fungi-technophyls: Biodestructive and corrosive agents

- Aspergillus niger
- Aspergillus flavus
- Penicillium chrysogenum
- Penicillium aurantiogriseum
- Cladosporium herbarum,
- Cladosporium cladosporioides

OCCURRENCE OF VARIOUS BACTERIAL GENES IN THE "MIR" ENVIRONMENT

OCCURRENCE OF VARIOUS FUNGAL GENES ON "MIR"

MICROBIAL CONTENT OF THE “MIR” AIR

lg CFU/m³

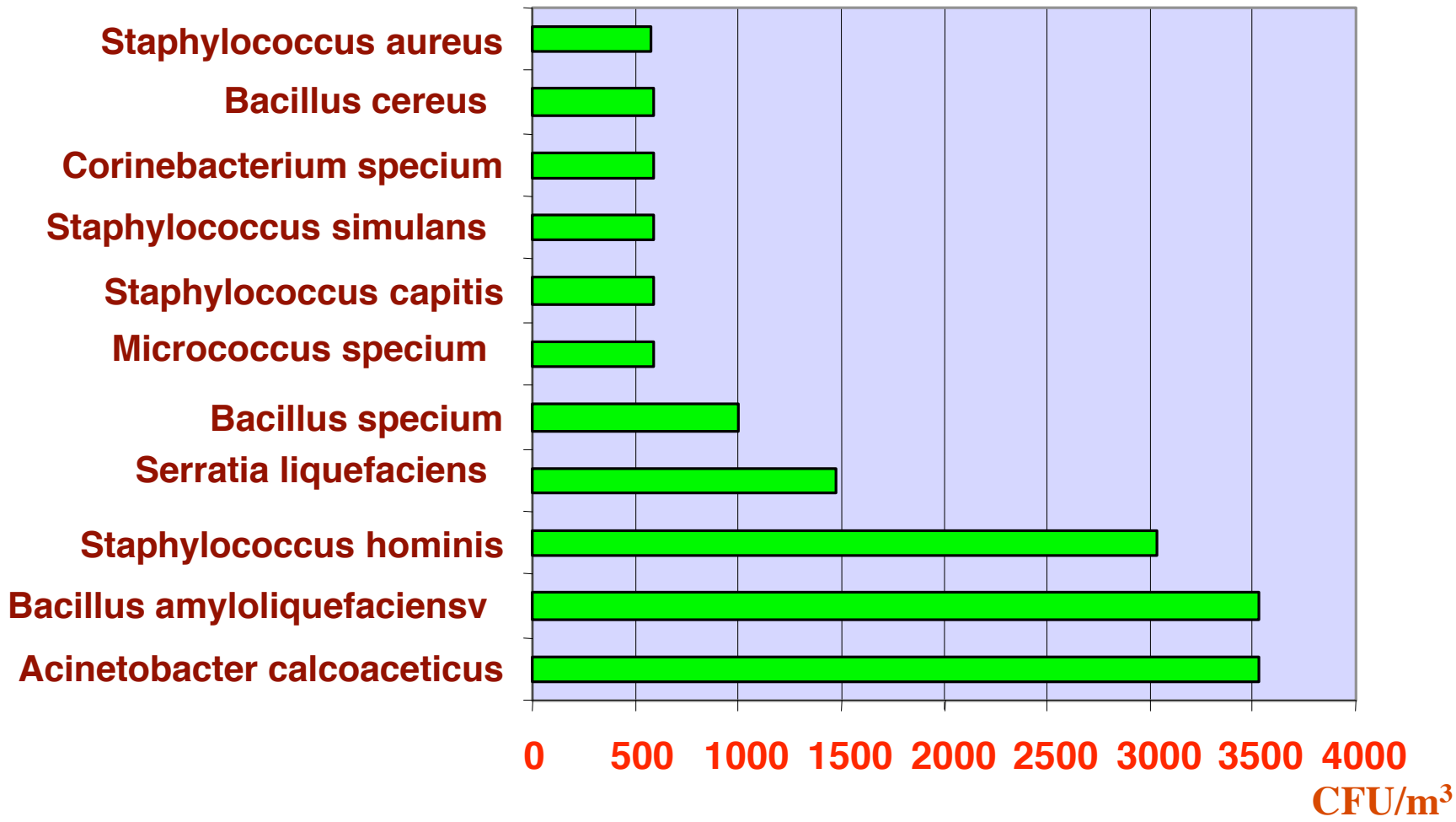
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
1,0E+00

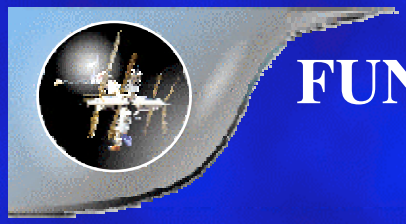
Fungi
Bacteria

ÝÎ -2
ÝÎ -4
ÝÎ -6
ÝÎ -8
ÝÎ -10
ÝÎ -12
ÝÎ -14
ÝÎ -16
ÝÎ -18
ÝÎ -20
ÝÎ -22
ÝÎ -24
ÝÎ -26

Missions

SPECIA OF AIR BACTERIA WITH CONCENTRATIONS IN EXCESS OF THEIR LIMITS

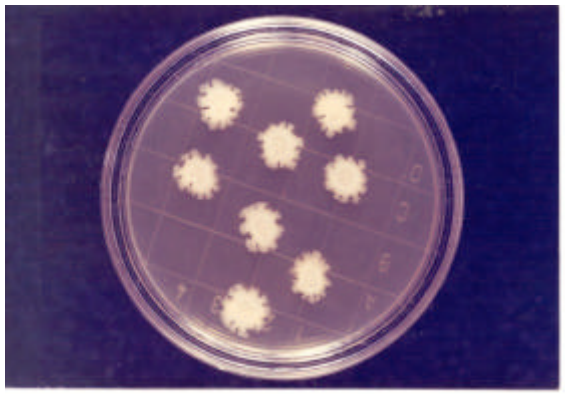




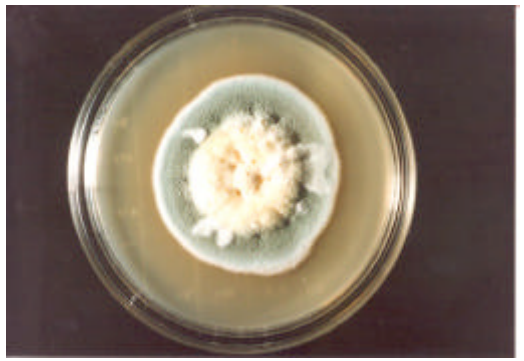
FUNGAL SPECIA WITH AIR CONCENTRATIONS IN EXCESS OF THEIR LIMITS

	CFU in 1 m^3	FUNGAL SPECIES
1	10 000	Sporobolomyces salmonicolor
2	1 000	Rhodotorula glutinus
3	600	Aspergillus thomii
4	464	Penicillium expansum
5	462	Aspergillus sp. from group A. versicolor
6	361	Penicillium sp.
7	280	Aspergillus versicolor
8	224	Penicillium verrucosum
9	215	Aspergills niger
10	165	Penicillium decumbens
11	140	Penicillium brevicompactum

BACTERIAL CONTENT OF THE "MIR" AIR



FUNGI CONTENT OF THE "MIR" AIR



MICROBIAL CONTENT OF THE "MIR" SURFACES

lg CFU/cm²

1,0E+08

1,0E+06

1,0E+04

1,0E+02

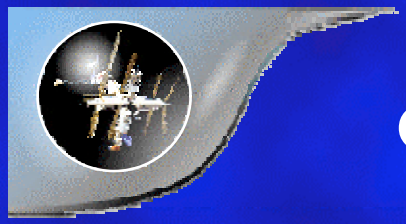
1,0E+00

Fungi

Bacteria

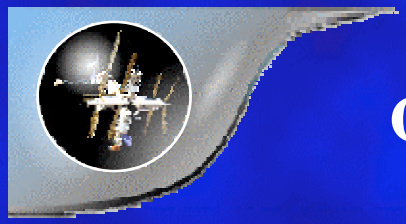
ÝÎ - 3 ÝÎ - 5 ÝÎ - 7 ÝÎ - 9 ÝÎ -11 ÝÎ -13 ÝÎ -15 ÝÎ -17 ÝÎ -19 ÝÎ -21 ÝÎ -23 ÝÎ -25 ÝÎ -27

MISSIONS



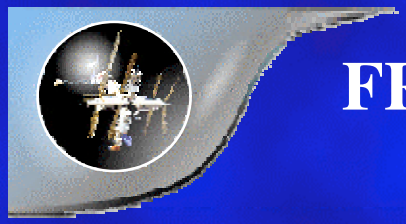
MAXIMAL LEVELS OF CONTAMINATION OF THE "MIR" INTERIOR AND EQUIPMENT BY VARIOUS BACTERIAL SPECIA

CFU in 100 cm^2	SPECIA OF BACTERIA
0 - 10^2	Aerococcus sp., Arthrobacter pyridinolis, Bacillus firmus, B. lignefaciens, B. striatum, Chryseomonas luteola, Corynebacterium aquaticum, C. ovis, Enterobacter sp., Hafnia alvei, Micrococcus roseus, Pasteurella haemolytica, Pseudomonas putida, Sarcina sp., Staphylococcus sciuri, Vibrio alginolyticus
10^2 - 10^5	Aeromonas caviae, A. hydrophila, A. veronii, Bacillus cereus, B. coagulans, B. licheniformis, B.macerans, B. pasteurii, B. polymyxa, B. pumilus, B. sp., B. thuringiensis, Corynebacterium bovis, C. equi, C. pseudodiphtheriticum, C. striatum, C. xerosis, Enterobacter agglomerans, E.cloacae, Kingella kingae, Klebsiella pneumoniae, Micrococcus varians, Moraxella sp., Neisseria sp., Pseudomonas stutzeri, Serratia fonticola, S. marcescens, Staphylococcus capitis, S. hominis, S. simulans, Streptococcus sp., Streptoverticillium sp., Xanthomonas maltophila
10^5 - 10^7	Acinetobacter calcoaceticus, A. sp., Actinobacillus ureal, Actinomyces sp., Alcaligenes faecalis, A. sp., Bacillus alvei, B. circulans, B. megaterium, B. simulans, B. sphaericus, B. subtilis, Corynebacterium sp., Enterobacter aerogenes, Escherichia coli, Haemophilis parainfluenzae, Micrococcus luteus, M. sp., Proteus sp., Pseudomonas paucimobilis, Serratia liquefaciens, S. p., Staphylococcus aureus, S. auricularis, S. cohnii, S. epidermidis, S. haemolyticus, S.aprophyticus, S. sp., S. warneri, S. xylosis, Streptomyces sp.



MAXIMAL CONTAMINATION OF THE "MIR" INTERIOR AND EQUIPMENT BY VARIOUS FUNGAL SPECIA

CFU in 100 cm^2	FUNGAL SPECIA
0 - 10^2	Alternaria alternata, Aspergillus biplanus, A. glaucus, A. ochr?ceus, A. spinulosus, A. unguis, ?????? A. wentii, Aureobasidium bollevi, Botryotrichum sp., Botrytis sp., Chaetomium elatum, Ch. globosum, Ch.sp., Fusarium moniliforme, F. sp.,Geotrichum candidum, G. flavo-brunneum, Lipomyces sp., Mucor pusillus, M. ranosissimus, M. sinensis, Penicillium arenicola, P. atramentosum, P. canescens, P. digitatum, P. diversum, P. grabrum, P.herquei, P. implicatum, P. jaczewskii, P. olivicilor, P. paxilli, P. pseudostromaticum, P. purpurogenum, P.simplicissimum, Rhodotorula rubra, Scopulariopsis sp.
10^2 - 10^5	Acremonium vitis, Arthrobotrys sp., Aspergillus candidus, A. clavatus, A. flavus, A. foetidus, A. fumigatus, A.ornatus, A. sp., A. sydowii, A. terreus, ?????? A. glaucus, Candida famata, C. sp., Cladosporium elatum, Cl.herbarum, Cl. tenuissimum, Cryptococcus neoformans, Cr. uniguttulatus, Mucor heterosporum, M. sp., Paecilomyces puntonii, P. sp., P. variotii, Penicillium camemberti, P.citreoviride, P. citrinum, P. crustosum, P.decumbens, P. echinulatum, P. granulatum, P. griseoroseum, P. hirsutum, P. megasporum, P. oseopurpureum, P. rugulosum, P. steckii, P. velutinum, Rhodotorula sp., Saccharamyces cerevisiae, Saccharamyces sp., Stemphylium botryosum, Sporobolomyces salmonicolor, Ulocladium botrytis
10^5 - 10^7 and higher	Acremonium charticola, A. roseum, A. sp., A. strictum, Aspergillus niger, A. versicolor, ?????? A. versicolor, Candida quillermondi, C. parapsilosis, Cladosporium cladosporioides, Cl. macrocarpum, Cl. oxysporum, Cl.sp., Cl. sphaerospermum, Cryptococcus laurentii, Paecilomyces lilacinus, Penicillium aurantiogriseum, P.brevicompactum, P. chrysogenum, P. Corylophilum, P. expancum, P. fagi, P. griseofulvum, P. italicum, P.puberulum, P. roqueforti, P. sp., P. spinulosum, P. Verrucosum, P. viridicatum, Rhodotorula glutinus, Scopulariopsis brevicaulis, Trichosporon pullulans, Tr. sp, Yarrowia lipolytica

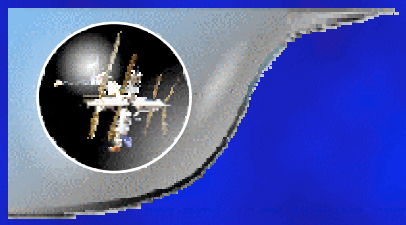


FREQUENCY OF DETECTION OF VARIOUS SORTS OF MICROORGANISMS (%)

Sort of microorganisms	System regeneration of water									
	SRW-C							SRW-W		
	model of station		Orbital station MIR					model of station		
			Mission 2-10		Mission 11-27					
	Con-den-sate	Water	Con-den-sate	Water	Con-den-sate	Water		Wetting	Conden-sate	Water
						Hott	Cold			
Acinetobacter	-	-	-	-				-		-
Aeromonas	-	-	-	20			14	-		-
Alcaligenes	-	-	-	40	15	15	21	-		-
Bacillus	-	-	-	-	3,7	5	7	-		-
Citrobacter	50	33	-	40	7,4	20	7	7	15	7
Clostridium	-	-	30	60	11	5	7	-		-
Enterobacter	75	67	10	20	7,4	15	7	-		-
Hafnia	-	16	-	-				-		-
Klebsiella	41	16	-	40				7	23	7
Moraxella	-	-	-	-			7	-		-
Pseudomonas	66	50	-	10	18	15	7	23	30	23
Proteus	-	-	-	-	7,4	10		38	76	38
Staphylococcus	75	66	100	70	100	75	78	42	57	30
Streptococcus	-	-	-	-				69	76	53

PRODUCTS OF A FEED

MICROBIOLOGICAL RISKS IN SPACE FLIGHT



Microbiological risks in space flight

Medical

Microflora of crew members

- Dysbacterioses
- Autoinfections
- Cross-infections

Appearance of independent sources of infection in the environment, reservoirs of potential pathogenic microorganisms

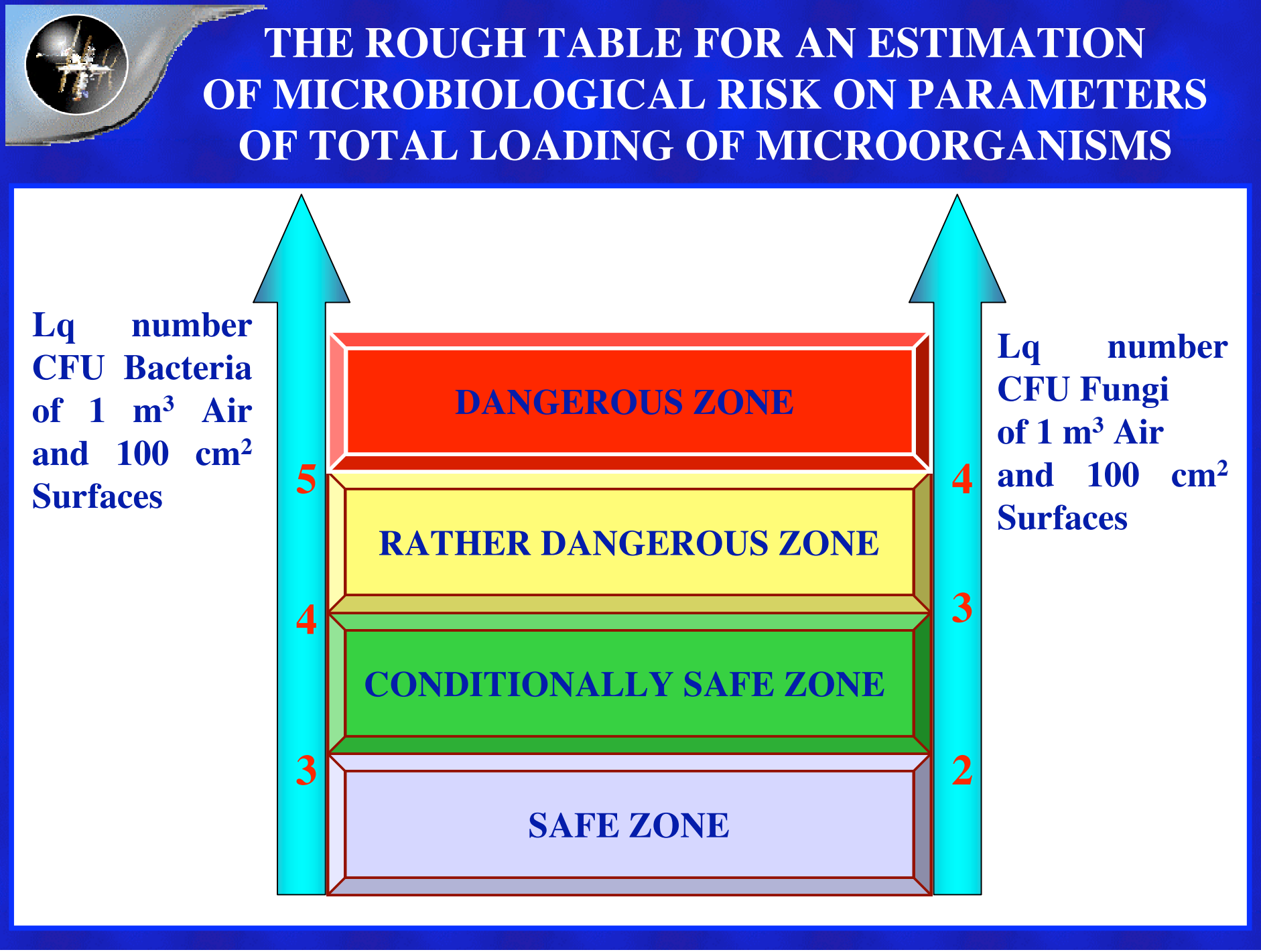
Technical (technological)

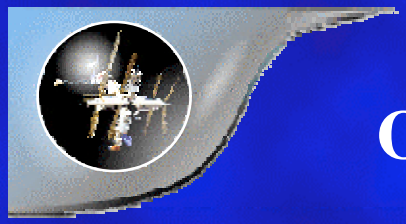
Microflora of the environment

Resident colonization of structural material of the interior and equipment in pressurized modules and LSS components by potentially pathogenic bacteria and fungi and fungi-biodegradersof polymers and agents of metallic corrosion

- Direct or indirect biodegradation of polymers
- Initiation of corrosion of metals
- Formation of biofilms on water of LSS and other systems
- Biointerferance, failure and malfunctioning of items and equipment

THE ROUGH TABLE FOR AN ESTIMATION OF MICROBIOLOGICAL RISK ON PARAMETERS OF TOTAL LOADING OF MICROORGANISMS

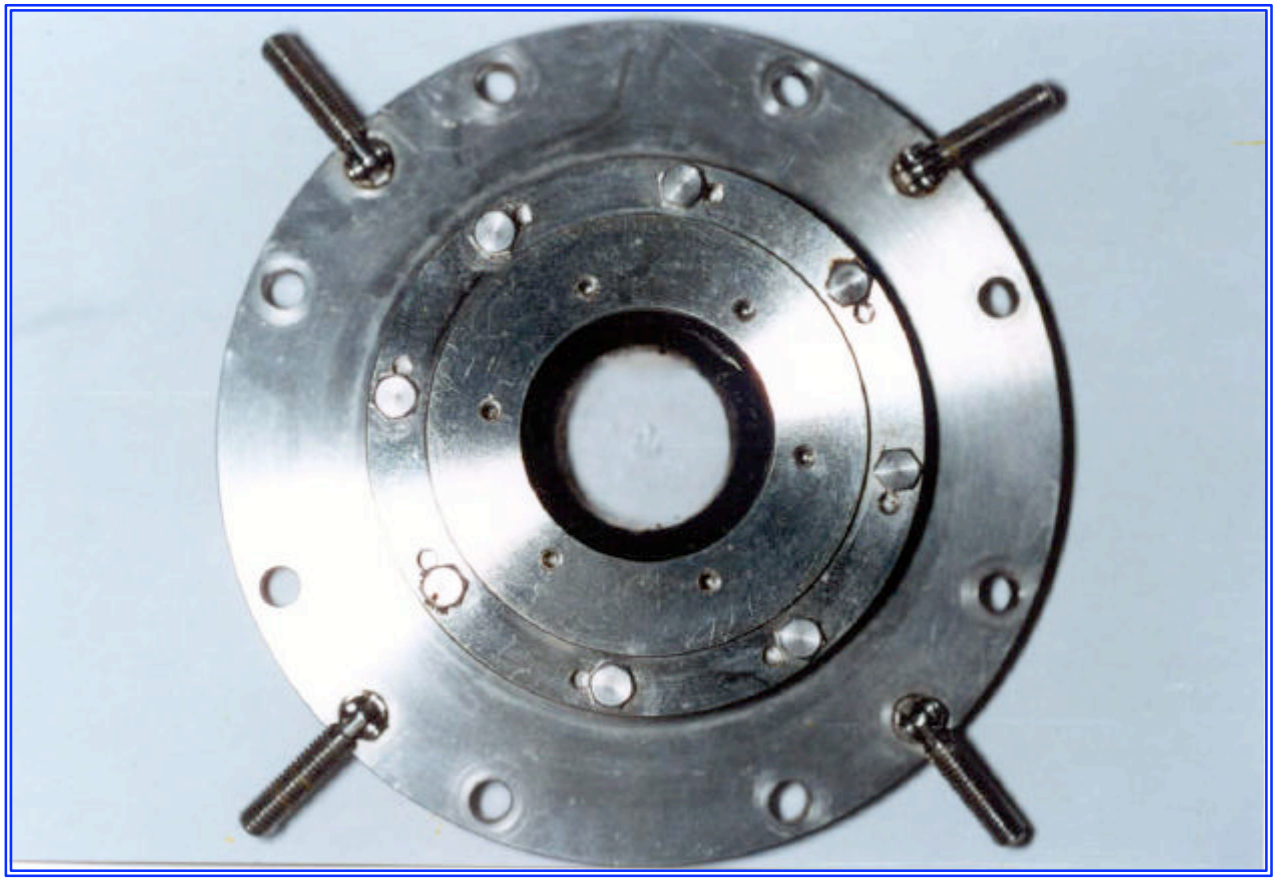




FACTS OF MICROBIAL DEGRADATION OF STRUCTURAL MATERIALS ON BOARD THE ORBITAL STATIONS

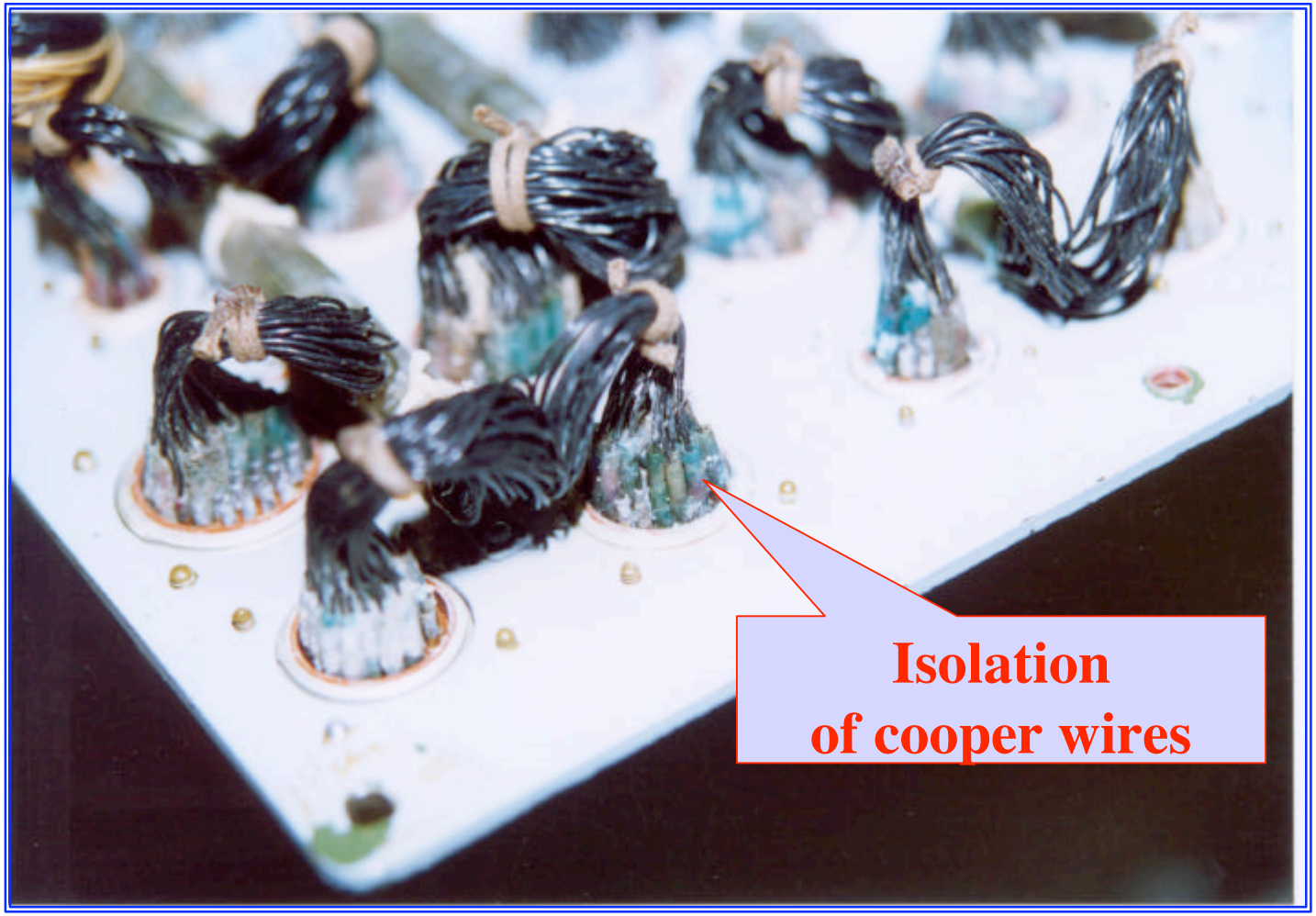
Orbital station, mission	Area of the station, equipment, outfit or material	Phenomenon
Salyut 6 main crew 5	Tubing and assemble	Visible growth of mold fungi in separate locations
Salyut 7 main crew 5	Sheathing , electrical connectors, cables	Visible growth of mold fungi.
Mir Main crew 3	Navigation window	Progressing decline of the window optics
Main crew 4	Components of the board air condensing unit, cables, surface of the freezer/dryer	Visible growth of mold species of varying pigmentation
Main crew 5	Oxygen electrolyzing unit	Zones with dense cover of mold fungi
Main crew 6	Components of EVA spacesuit	Visible growth of mold fungi
Main crew 8	Sheathing close to the toilet and the control station	Visible growth of micromycets
Main crew 11 -15	Thermal control system, tubing WRS-U components, WRS-C air conditioner	Visible growth of mold fungi, Repeated malfunctioning of the systems caused by gel-like thrombi in water ducts along which condensate goes to regeneration.
Main crews 16 –17	Video and still cameras, insulation of cables	Visible growth of mold fungi in several locations
Main crews 19 – 22	Thermal control ducts, sheathing, WRS-C, surfaces behind panels tubing and the casing	Visible growth of mold fungi, corrosion of metals
Main crew 23	Basal module.WRS-C, tubing, surfaces behind panels	Visible growth of mold fungi, seats of corrosion and cavern (up to 2mm)
Main crew 24	External surface of the EVA spacesuit, surfaces behind panels, sheathing, communication control unit	Visible growth of mold fungi
Main crews 25 – 27	Shell of the pressurized module, navigation window	Areas of visible growth of mold fungi on frames, TCS, insulation tubes, behind panels, rubber spacers of the hatches; metallic corrosion

CONTAMINATION OF WINDOW BY MOLD FUNGI IN LABORATORY EXPERIMENT



GROWTH OF MOLD FUNGI ON THE COMMUNICATION DEVICE WHITE AND BLACK TUBES

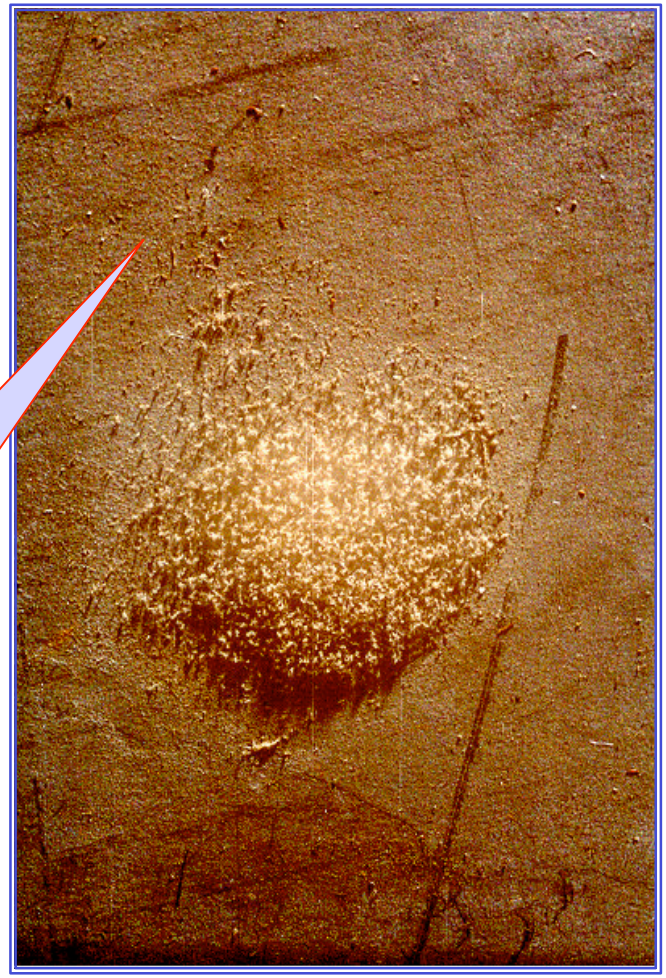
GROWTH OF MOLD FUNGI ON THE COMMUNICATION DEVICE INSULATION BLOCK



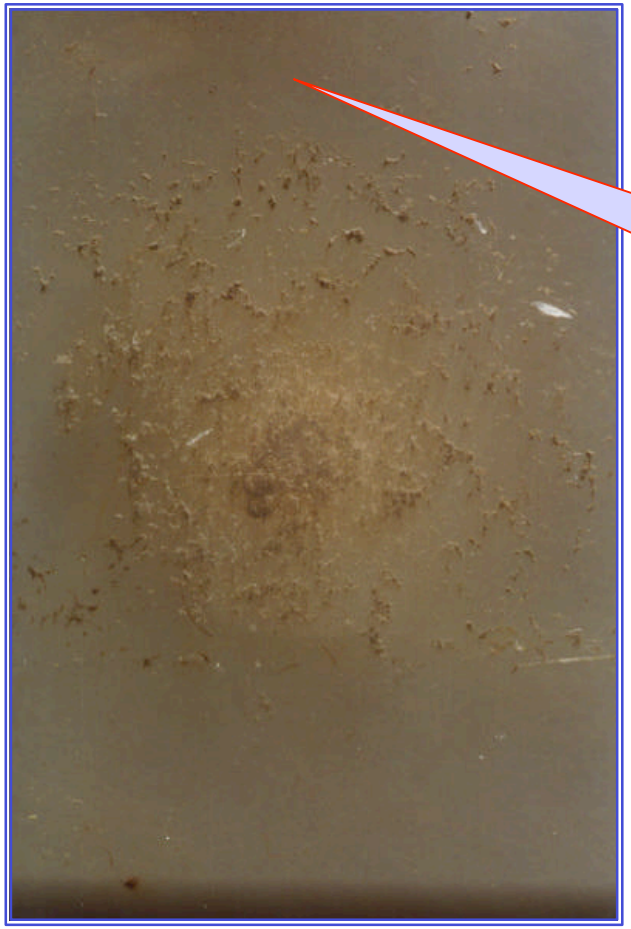
MATERIALS BIODEQRADATION

RUBBER

TITANIUM

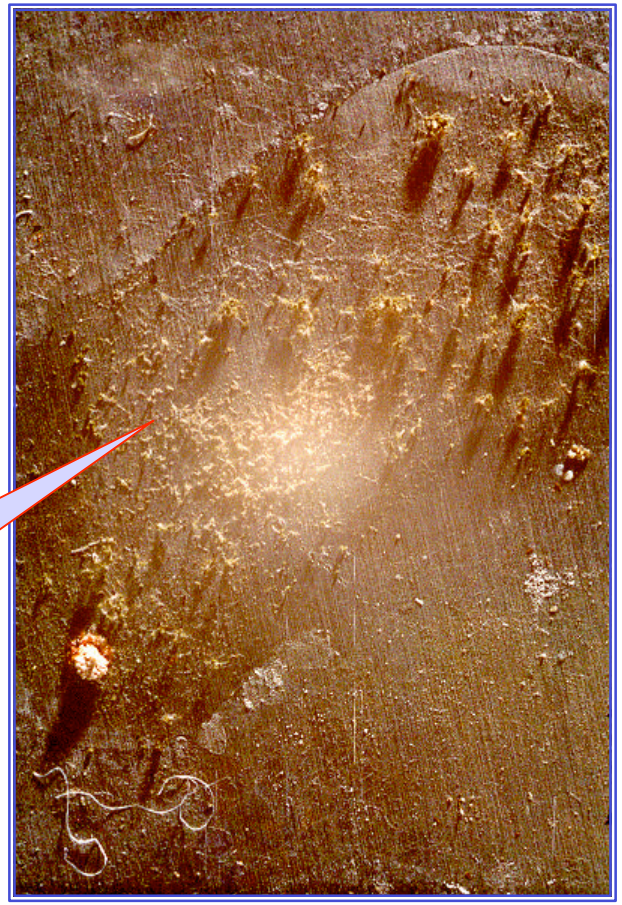


MATERIALS BIODEQRADATION

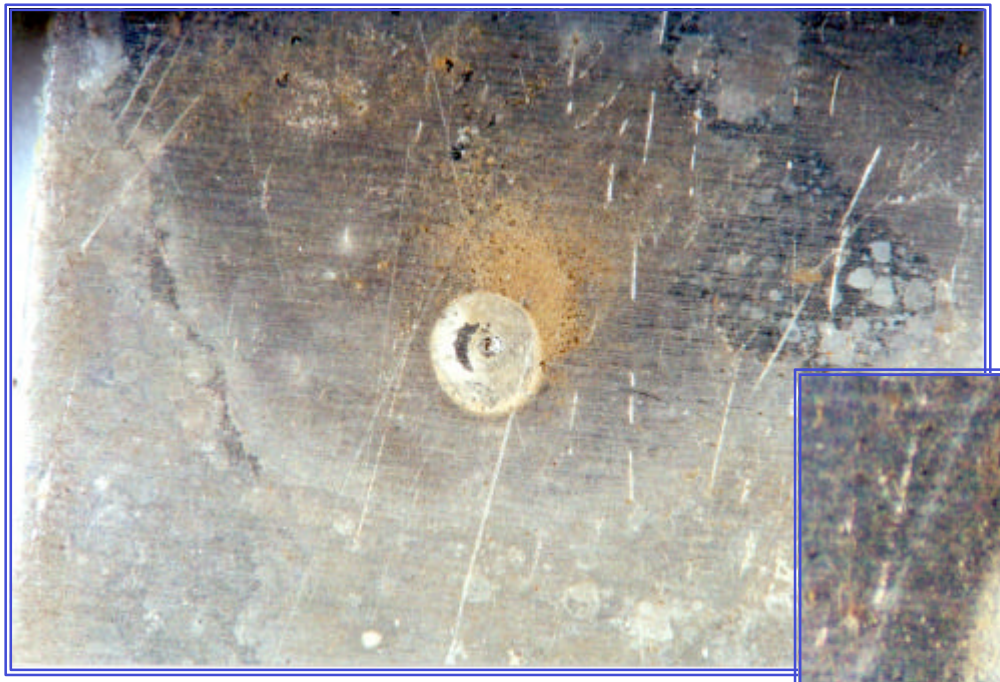


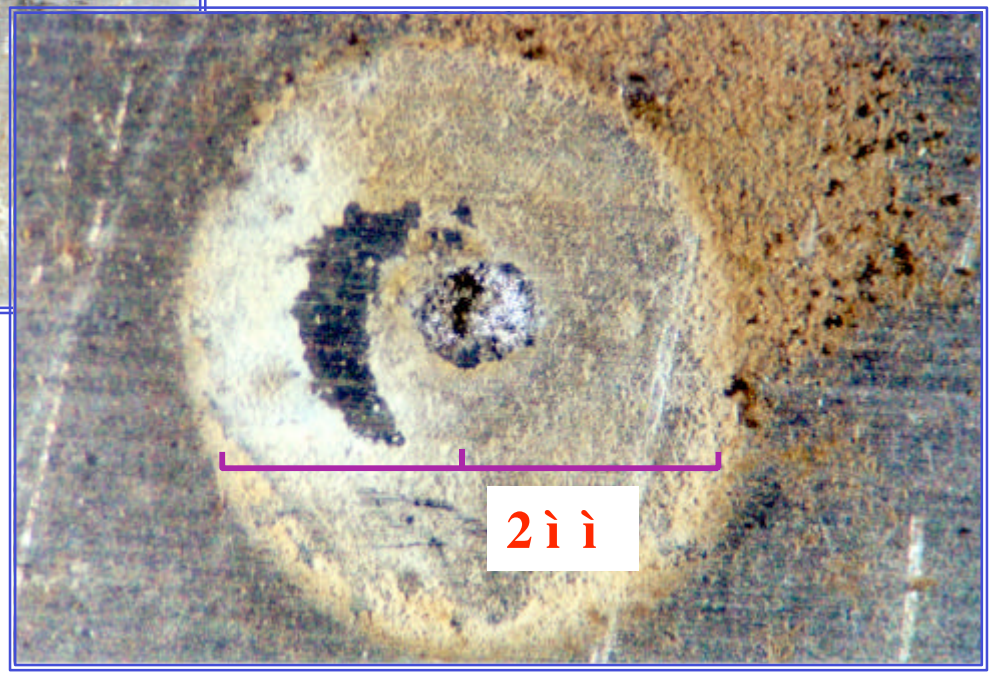
ELECTRICAL TAPE

ALUMINIUM



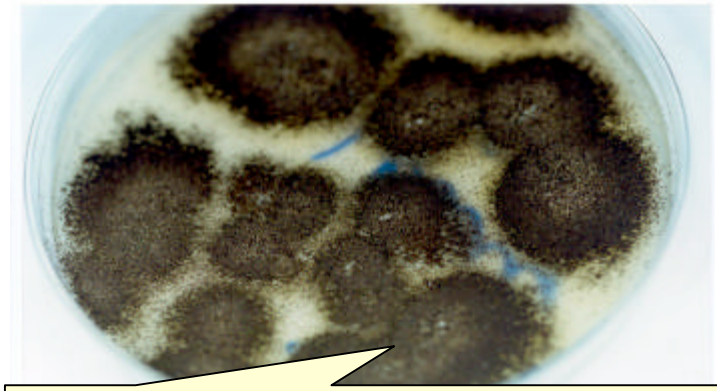
DAMAGES ALIMUNUM





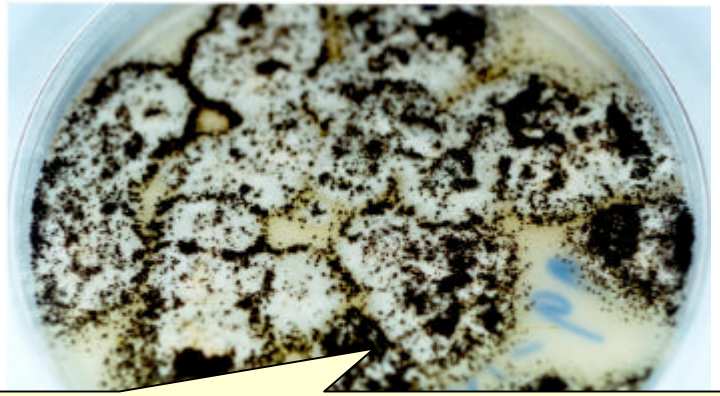
GROWTH OF FUNGI ASPERGILLUS VERSICOLOR, ASPERGILLUS NIGER SP. DURING CHRONIC γ-IRRADIATION AND CONTROL

velvety-felt surface of colonies



Culture is in the state of normal spore-formation

Puffy surface of colonies with well-observed colonies of white vegetative mycelium in the center

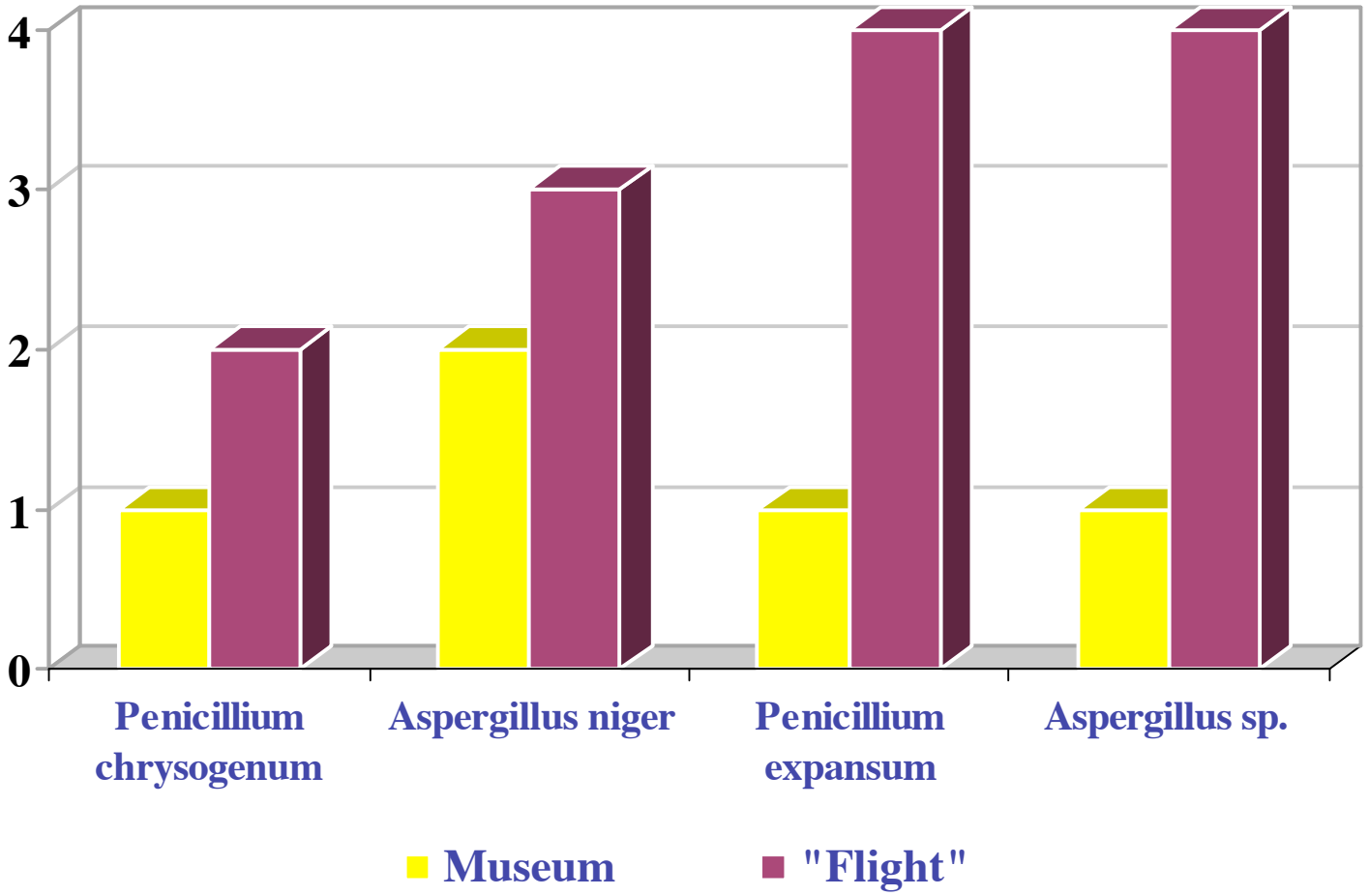


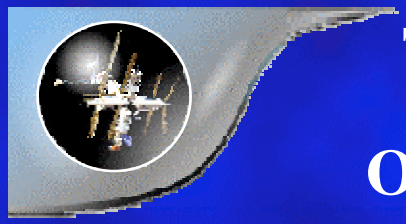
Expansion of vegetative mycelium misshaping the fungal colony

EVOLUTION OF STRUCTURE FUNGI – CHANGE OF DOMINANT KINDS OF FUNGI IN MIR DURING LONG-TERM OPERATION

Missions	2	3	4	5	6	7	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Penicillium chrysogenum	■	■	■	■	■	■			■	■	■	■	■	■	■								
Penicillium griseoroseum	■																						
Aspergillus niger		■		■		■											■	■	■	■			■
Cladosporium herbarum			■																				
Penicillium aurantiogriseum					■				■									■	■	■	■		
Paecilomyces variotii						■																	
Penicillium velutinum							■																
Aspergillus versicolor				■		■	■									■	■	■	■	■	■	■	■
Penicillium viridicatum								■										■	■	■			
Penicillium griseofulvum																■							
Penicillium expansum																■	■	■	■	■	■	■	■
Cladosporium cladosporioides et ñl.sphaerospermum								■	■							■	■	■	■	■	■	■	■
Yarrowia lipolytica																	■	■	■	■			

AGGRESSIVENESS OF FUNGAL STRAINS RECOVERED FROM MIR AS COMPARED WITH REFERENCE (MUSEUM) CULTURES





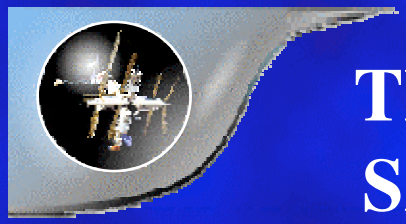
THE FOLLOWING SUPPOSITIONS CAN BE MADE TO CHARACTERIZE EVOLUTION OF THE MICROBIAL COMMUNITY ABOARD LONG-OPERATING SPACE VEHICLE

- **Environment of a long-operating piloted space vehicle may be a peculiar kind of ecological niche for development and reproduction of bacilli and fungi belonging to particular species;**

- **bacteriofungal associations primarily reside on decorative-finish and structural materials of space interior and equipment which gather anthropogenic organic compounds and air condensate enough to allow full vegetative cycle and reproduction of heterotrophic microorganisms, mold fungi Penicillium, Aspergillus, Cladosporium sp. in the first place;**

- **quantitative and structural dynamics of microflora on long-operating space vehicles is not linear and presents a wave-form cycle of alternating phases of biocenosis activation and stagnation controlled as by internal biological mechanisms of self-regulation, so by external cosmophysical factors;**

- **the phase of microflora activation is fraught with medical and technical risks that can significantly impact flight safety and hardware reliability.**



THE SYSTEM TO SECURE MICROBIAL SAFETY OF ORBITAL STATION "MIR"

Crew anti -microbial measures

- Comprehensive microbiologic and immunology investigation
- Restrained social contacts and observation regimen in the pre launch period
- Use of personal hygienic means medical kits

Antimicrobial measures applied to the space station modules and LSS components, transport vehicles and deliveries

Preflight

- Establishment of microbial requirements to water, foods, gaseous environment, and interior of habitable modules of space station and transport vehicles
- Testing of decorative and structural materials for microbial resistance
- Implementation of the biologic cleanness requirements during assembly, outfitting and pre-launch treatment of the compartments of transport and cargo vehicles, and deliveries
- Pre-launch disinfection of space modules and vehicles, collection and analysis of reference microbial samples

In flight

- Periodic collection and analyses of microbial samples of air, atmospheric condensate, and surfaces structural materials
- Cleanup of habitable compartments with the use of antimicrobic means, treatment with Fungistat to stop microbial degradation
- Decontamination of the gaseous environment by the POTOK unit
- Pasteurization of regenerated water before
- Inspection of the interior and equipment in the habitable compartments in order to detect spots with signs of microbial degradation

THE PRIORITIZED DIRECTIONS OF PERFECTION OF METHODS AND MEANS OF MICROBIOLOGICAL SAFETY IN LONG-OPERATING SPACE VEHICLES

Scientific researches

- chanisms of self-regulation of biotechnocenoses appearing in the environment of long-operating space vehicles;
- external factors-inductors of anabiosis and initiators of metabolic activity in microorganisms specific for the spaceflight conditions;
- margins of the phenotypic adaptation and genotypic changeability of microorganisms residing in long-term operating space vehicle.

Practical development

- approaches to modification of surface of materials that will protect against biocontamination and increase resistance to microorganisms;
- instruments and tools for all-out checkup and diagnostics of early phases of biodegradation, and blocking biodegradation and biocorrosion;
- board test-systems to investigate phenotypic and genotypic changeability of microflora in space flight including the dissociative potential of resting forms.